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Original Article

Clinico-mycological study of onychomycosis

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ABSTRACT

Background: Onychomycosis is a common fungal infection of the nails caused by dermatophytes, yeasts and non-dermatophytes molds. It leads to nail discoloration, thickening and represents an important public health concern due to its chronic nature, recurrence and impact quality of life. **Objective:** To study the clinical and mycological features of onychomycosis and their relation to age and sex. **Materials and methods:** One hundred and thirty-five patients with the clinically suspected diagnosis of onychomycosis attending, dermatology department Benghazi- Libya over a period of one year (2023-2024). All cases were carefully examined including detailed disease history and complete dermatological examination. Nail samples were collected for mycological tests including direct microscopic examination using potassium hydroxide (KOH) 20%and culturing on Sabouraud's dextrose agar, containing cycloheximide and chloramphenicol. **Results:** Out of a total of 135 patients, 119 patients were diagnosed with onychomycosis including 96 (80.7%) females and 23 (19.3%) males. Finger nail fungal infections were more common than toe nail infections (65.5%) and (18.5%) respectively, and significant increase in incidence of finger nail onychomycosis among females than males 74% and 30.4% respectively. distal lateral subungual onychomycosis (DLSO) was predominant clinical type of onychomycosis in both fingernails and toenails (42.3%) and (45.5%) respectively. Direct microscopic examination was positive in 37% of the patients, and both direct microscopic and culture was positive in 37.8% of patients. Both Candida and non-dermatophyte molds (NDMs) were the most common pathogens isolated, (37%) followed by dermatophytes (26%). **Conclusions:** onychomycosis was more common among females with fingernail involvement predominating. The DLSO type was the most common clinical pattern. Candida albicans, Aspergillus nigra and Trichophyton Tonsurans were the main causative agents.

Key words: Onychomycosis, Clinical types, Non-dermatophytes, Yeast, Non-dermatophyte molds, Dermatophytes

INTRODUCTION

Onychomycosis is a fungal infection of nails caused by dermatophytes, non-dermatophytes and yeasts.it affects approximately 5% of the population worldwide [1] and represents around 30% of all superficial fungal infection [2] and 50% of nail disorders [3].

Onychomycosis is often primarily a cosmetic problem, but it is actually a debilitating disease. It has negative physical and psychological effects on the patient. Therefore, it is very important to treat fungal nail diseases [4-6].

Nail changes in onychomycosis can occur in various forms onychodystrophy, onycholysis, subungual hyperkeratosis and discoloration or thickening of nail plate [7].

The fingernails onychomycosis is commonly seen in females as they more likely exposed to water and chemical detergents during daily domestic work whereas toenail infections was commoner in males due to prolonged use of closed footwear and increased physical activity [8].

The clinical presentations of onychomycosis are Distal Lateral Subungual Onychomycosis (DLSO), Proximal Subungual Onychomycosis (PSO), White Superficial Onychomycosis (WSO), and Total Dystrophic Onychomycosis (TDO) [9].

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Direct microscopy using KOH preparation plays an important role in diagnosing nail fungal infections. However, fungal culture is the only definitive test that can be used to identify the species of the infectious organism [10].

Fungi causing onychomycosis to vary with the geographical distribution and differ from one country to another according to temperature and lifestyle of these countries [10,11].

Dermatophytes particularly Tricophyton rubrm and Trichophyton mentagrophytes represent 90% of toenail fungal infection and 50% of fingernail onychomycosis [12].

Candida species are second in frequency to dermatophytes as causative agents of onychomycosis. They constitute 10%-32% of toenail onychomycosis and 51%-70% of fingernail infections [13].

Non-dermatophyte molds are saprophytic non keratinolytic fungi that live in the soil and may be primary or secondary pathogens of the skin and nails. Previous nail destruction by a dermatophyte, trauma, or another nail disease favors nail infection by NDMs. The widespread use of broad-spectrum antibiotics, corticosteroids and other immunosuppressive drugs increase the incidence of nail infection due to NDMs [14].

MATERIALS AND METHODS

Study Design

A cross-sectional study was conducted including all patients with confirmed diagnosis of onychomycosis who attending dermatology department Benghazi -Libya over a period of one year (2023-2024). All patients provided verbally informed consent to participate. Each participant was carefully examined, including detailed history and complete dermatological examination.

Sample Size

The sample was obtained conveniently based on the frequency of all patients attending the dermatology department during the study period and was estimated to be 135 patients.

Inclusion Criteria

Patients of both genders and different age groups who presented clinically suspected onychomycosis and agreed to participate in the study were included.

Exclusion Criteria

Patients who received systemic antifungal therapy within the last four weeks or topical antifungal therapy within the last one week. Patients who refused participated in the study.

Sample Collection

After obtaining verbal consent from all participants, nail samples were collected directly from each patient by clipping the affected nail after cleaning the area with 70% alcohol. A new sterile nail clipper was used for each patient to prevent cross- contamination. Samples were labeled and divided into two portions: one for direct microscopic examination and the other for fungal culture.

Direct Microscopic Examination

The collected specimen was placed in a test tube, and a few drops of 20% potassium hydroxide (KOH) solution were added using an eye dropper. The tube was then kept for 24 hours to dissolve the keratin. The collected specimen was mounted on glass slide and covered with a cover slip. Repeated KOH examination was performed before the specimen was considered negative for direct microscopic mount.

Cultivation of the Specimens

The specimen of each patient was placed in separate sterile Petri dish. Each specimen was inoculated on sabouraud`s dextrose Agar (SDA), and Fung biotic agar. The inoculated plates were kept in the incubator which was adjusted at 28°C and the cultures were examined every two days. The culture was considered negative if there was no growth after four weeks of incubation.

The positive specimens (fungi cultures) were mounted with the lactophenol cotton blue to reveal various structures which could be of great help in identification, especially the conidia which include the large separated macroconidia and the small celled microconidia. The macroconidia of each genus and species vary in

shape and character of their walls which are generally characteristic for the species or genus.

The identification of Candida species was based on the presence of budding cells and pseudohyphae. The BD PHOENIX (Becto, Dickinson/USA) is an automated microbiology system intended for the in vitro rapid identification (ID) of yeast and yeast like organisms.

Statistical Analysis

Frequency tables and chart constructed for our data were analyzed statistically using the chi-square test. We assumed results are statistically significant when P value is < 0.005. The statistical analysis of the results was carried out according to the computer package (SPSS 18.0 version).

Ethics Statement

Ethical consideration included the fact that all participants provided verbal consent to participate in the study after being informed about its purpose and procedures. The study was conducted in accordance with the ethical principle of the declaration of Helsinki. The research was authorized by the institutional Ethics committee, and confidentiality of all participants' data was strictly maintained throughout the study. Data were used solely for scientific purposes.

RESULTS

Out of a total of 135 patients, 119 were diagnosed with onychomycosis based on culture and, or direct microscopic examination. Of these 119 patients 96 (80.7%) were females and 23 (19.3%) were males. The mean age of patients with onychomycosis was 40.7± 14.34years. Onychomycosis was most prevalent in the age 30-39 years age group, with a female: male ratio of 4.2:1 (p= 0.013) demonstrating statistically significant association between age and sex (Table 1).

Fungal culture was positive in 111 (82.2%) specimens (Table 2). Direct microscopic examination was positive in 50 (37%) specimens and negative in 85(63%) specimens and positive direct microscopic alone in 8 (33.3%) of cases (Figs 1a and 1b).

Fingernails were involved in 78 (65.5%) cases, toenails in 22 (18.5%) cases and both fingernails and toenails were affected in 19 (16%) cases. Fingernail involvement was statistically significant in women (p= 0.001) while toenail involvement was statistically significant in men (p= 0.001) (Table 3). Distal lateral subungual onychomycosis (DLSO) was the most common clinical type observed in 33 (42.3%) (Fig. 2a) in fingernails and 10 (45.5%) in toenails, followed by proximal subungual onychomycosis (PSO) in 24 (30.8%) of fingernails (Fig. 2b) and 6 (27.3%) of toenails.

Table 1: Age and sex distribution among patients with onychomycosis

Age group (years)	Male n (%)	Female n (%)	Total N (%)
10–19	2 (8.7)	1 (1.0)	3 (2.5)
20–29	5 (21.7)	18 (18.8)	23 (19.3)
30–39	4 (17.4)	32 (33.3)	36 (30.3)
40–49	3 (13.0)	23 (24)	26 (21.8)
50–59	2 (8.8)	15 (5.7)	17 (14.3)
60–69	4 (17.4)	5 (2.5)	9 (7.6)
70–79	2 (8.7)	2 (2)	4 (3.4)
80–89	1 (4.3)	0 (0)	1 (0.8)
Total	23 (100)	96 (100)	119 (100)

(χ^2=17.82, df=7, p=0.013)

Table 2: The result of culture growth in relation to direct microscopic KOH examination

Direct microscopic examination	Result of culture	Total (N)	%
	Positive culture	Negative culture	
KOH positive	42 (37.8%)	69 (62.2%)	111
KOH negative	8 (33.3%)	16 (66.7%)	24
Total	50	85	135

Table 3: Distribution of nail involvement among patients with onychomycosis according to sex

Nail involvement	Male n (%)	Female n (%)	Total N (%)
Fingernail	7 (30.4)	71 (74.0)	78 (65.5)
Toenail	11 (47.9)	11 (11.4)	22 (18.5)
Both	5 (21.7)	14 (14.6)	19 (16.0)
Total	23 (100)	96 (100)	119 (100)

(χ^2 = 19.23, df=2, p < 0.001)

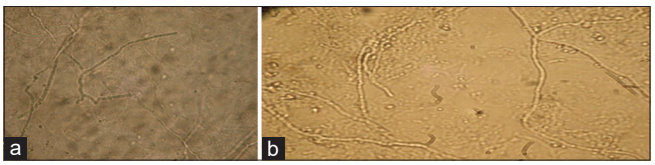

Figure 1: (a) Potassium hydroxide mount-showing hyphae. (b) Potassium hydroxide mount-showing septated hyphae.

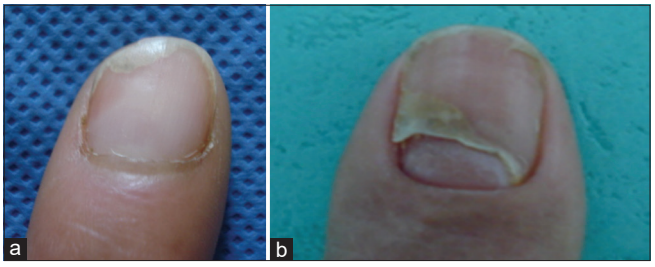

Figure 2: (a) Distal subungual onychomycosis. (b) Proximal subungual onychomycosis.

White superficial onychomycosis was observed in 11 (14.1%) in fingernails and 3 (13.6%) toenails (Fig. 3a). total dystrophic onychomycosis was observed in 10 (12.8%) in fingernails (Fig. 3b) and 3 (13.6) in toenails (Table 4). Candida and non-dermatophyte molds were the most frequent causative agents in 37% each (Table 5).

Among yeasts were the common causative agents isolated *Candida albicans* (Figs 4a and 4b) was observed in 19 (46.3%) followed by Candida *parapsilosis complex in 15(36.7%)*, and *Candida tropicalis observed in 7 (17%)*.

Among non-dermatophyte molds, Aspergillus nigra (Figs 5a and 5b) was the most common isolate observed in 15 (36.7%), followed by *Penicillium spp.* (Figs 6a and 6b) in 7 (17.1%). Others non-dermatophytic strains were isolated Aspergillus flavus (14.6%), Fusarium spp. *(9.8%)*, *Scopulariopsis spp. (9.8%)*, *Alternaria spp. (7.3%)*, *Chaetomium spp.* (2.4%) and *Cladosporium spp. (2.4%)*.

Among dermatophytes were the common causative agents isolated Trichophyton tonsurans (Figs 7a and 7b) was observed in 10 (34.5%) followed by Trichophyton verrucosum in 7 (24%) (Figs 8a and 8b).

Other dermatophytic strains were isolated, include *Trichophyton violaceum (13.8%)*, *Trichophyton soudanense (Figs 9 and 9b) (10.3%)*, *Trichophyton mentagrophytes (7%)*, *Trichophyton rubrum (7%)*, and *Epidermophyton floccosum* (3.4%).

DISCUSSION

Onychomycosis is a chronic infection of the nails, and is considered a serious problem for public health [15]. Onychomycosis has both psychosocial and physical detrimental effects on quality of life [16]. In the present study, onychomycosis was found to be common in the age 30-39 years (30.3%). similar findings also reported in studies from Saudi Arabia, Indonesia and India [17-19]. In contrast, some studies have reported that onychomycosis affected elderly individuals [20]. The increase in incidence of onychomycosis in younger population could be explained by the fact that they are more often exposed to occupation related trauma that predisposes them to infections and they may also comparatively more cosmetically aware than older age group. This study revealed that there was

Table 4: Clinical Types of onychomycosis and sites of nail involvement

Type of nail Involvement	Finger nail 65.5% n = (78)	Toe nail 18.4% n = (22)	Fingernail and Toenail 15.9% n = (19)	Total N = (119)
DLSO	33(42.3%)	10(45.5%)	9(47.4%)	52
PSO	24(30.8%)	6(27.3%)	6(31.6%)	36
WSO	11(14.1%)	3(13.6%)	2(10.5%)	16
TDO	10(12.8%)	3(13.6%)	2(10.5%)	15

Table 5: Causative fungal agents isolated from patients with onychomycosis (N = 111)

Isolates	Species	Number	Percent (%)
Candida *(n = 41, 37%)*	*Candida albicans*	16	19.0
	Candida parapsilosis complex	13	15.0
	Candida tropicalis	6	7.0
Nondermatophytes *(n = 41, 37%)*	*Aspergillus niger*	15	36.6
	Penicillium spp.	7	17.1
	Aspergillus flavus	6	14.6
	Fusarium spp.	4	9.8
	Scopulariopsis spp.	4	9.8
	Alternaria spp.	3	7.3
	Chaetomium spp.	1	2.4
	Cladosporium spp.	1	2.4
Dermatophytes *(n = 29, 26%)*	*Trichophyton tonsurans*	10	34.5
	Trichophyton verrucosum	7	24.0
	Trichophyton violaceum	4	13.8
	Trichophyton soudanense	3	10.3
	Trichophyton mentagrophytes	2	7.0
	Trichophyton rubrum	2	7.0
	Epidermophyton floccosum	1	3.4

Figure 3: (a) White superficial onychomycosis. (b) Total dystrophic onychomycosis.

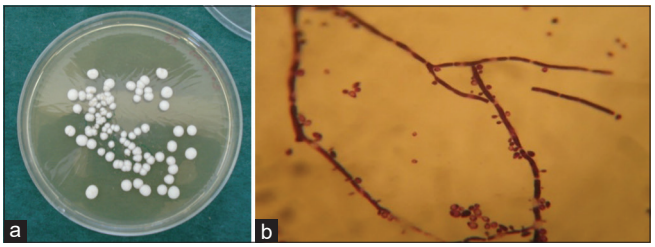

Figure 4: (a) candida albicanis colony. (b) blastoconidia and pseeudohyphae.

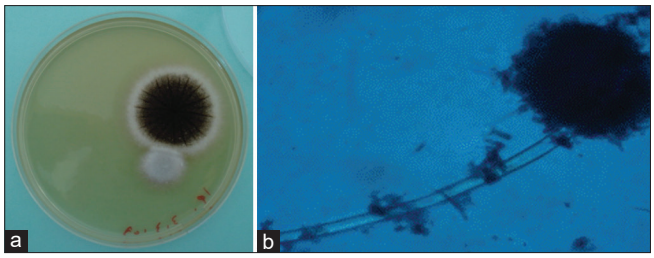

Figure 5: (a) Aspergillus nigra colony. (b) large spore black head, non-sepateted conidiophore.

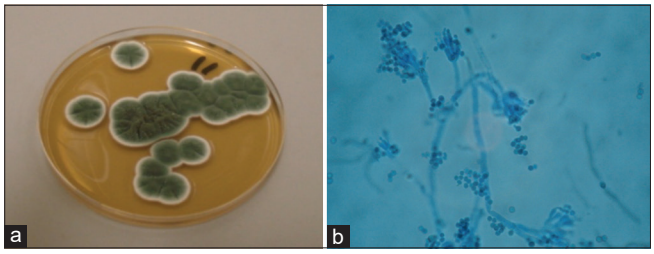

Figure 6: (a) Penicillium colony. (b) Branched conidiophores and conidia.

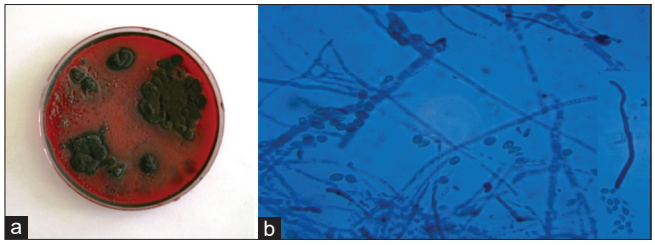

Figure 7: (a) Trichophyton tonsurans. (b) Balloon shaped microcondia and macroconidia.

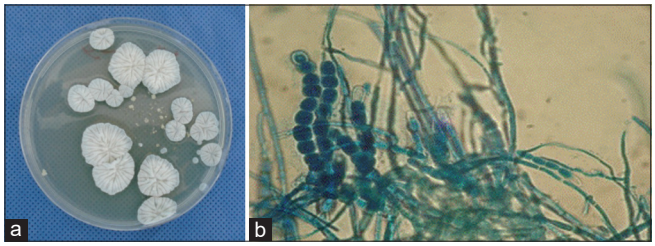

Figure 8: (a) Trichophyton verrucosum. (b) Chlamydospores forming in chains.

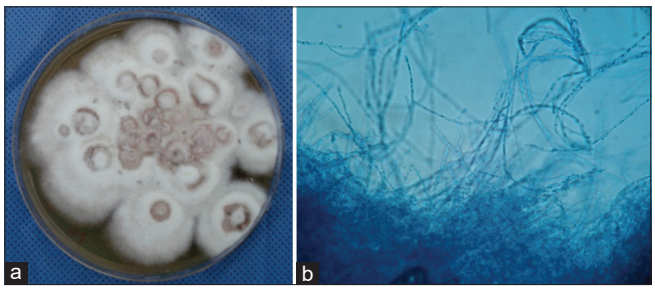

Figure 9: (a) Trichophyton soudanenes. (b) Hyphae with typical reflexive branche.

highly significant difference between male and female (p=0.001), that's mean the females more affected than males and the female to male ratio was 4.2:1. This result was consistent with other studies, which reported a higher incidence in female [21]. In contrast to other studies who had reported males to be affect more than females [19]. Fingernail fungal infections were more common than toenail infections (65.5% and 18.5% respectively) and significant increase in incidence of finger nail onychomycosis among females than males 74% and 30.4% respectively (p=0.001). This difference may be explained by the work habits of female patients who perform domestic chores in some occupations, such as: cooking, washing clothes and floor cleaning, with wet hands for most of the day. In addition, this state of affairs is aggravated by the chemical action of detergents. These causal factors are supported by the large incidence of infection of the fingernails in women (74%) compared to those of men (30.4%). This result was consistent with other studies, which reported a higher incidence in female [21-23].

Toe nail onychomycosis was more affecting male patients than female patients,47.9% and 11.4% respectively, and this difference was highly significant (p = 0.001). This could be explained by religious (Muslim) practice of washing the feet five times daily may be a possible rout of fungal infection of toenails (19), and the difference between male and female could be due to the difference between male and female in the life style and type of work, which lead to more toenail trauma in males that led to secondary fungal infection. This finding was reported with observation of P veer et al. who reported that toenails involvement was more frequent in males than females [24].

The most common variety of onychomycosis, distal lateral subungual onychomycosis (DLSO), is characterized by invasion of the nail bed and underside of the nail plate. The high incidence of DLSO pattern (33.6%) followed by PSO (23.5%), in current study was in accordance with other studies [19,25].

Direct microscopy using KOH preparation plays an important role in diagnosing nail fungal infections. However, fungal culture is the only definitive test that can be used to identify the species of the infectious organism [10]. In the present study, fungal culture growth was reported in 82.2% of cases while no growth was observed in 17.8% of cases. These results are relatively similar to those results reported by;

EL-Hamed et al. [26] who obtained positive culture for fungi in 80.9% of cases and no growth observed in 19.1% of cases.

In our study, yeast and non-dermatophyte molds were the most common causative agents each accounting for (37%) of cases while dermatophytes were isolated in (26%) of cases. Similar other studies have also reported yeasts and molds as the predominant pathogens involved in onychomycosis [23,27]. Among the yeasts, Candida albicans were the most common isolates in (46.3%) of cases followed by Candida parapsilosis complex in (15%) of cases. Similar findings reported in Tunisia [28], candida albicans accounted for (55.6%) and candida parapsilosis (8.2%) of candida nail infections. A high isolation rate of NADs was observed in current study (37%). Similar high the isolation in Malaysia (45.4%) and Teheran (40.5%) [10,14]. In contrast studies where the isolation rate of NDMs varied from (1.49%) and (33%.5) [29,30]. This variation may reflect geographic differences in molds distribution and humidity. In addition, use of broad-spectrum antibiotics, and immunosuppression drugs.

Among NDMs isolates, Aspergillus nigra was the commonest fungal isolate (36.6%), followed by Penicillium spp. (17.1%). Meanwhile in the study from Egypt, it has been reported that Aspergillus Niger is the commonest fungal isolates from nail disorder [31]. Other non-dermatophytic strains were isolated Aspergillus flavus (14.6%), Fusarium (9.8%) Scopulariropsis spp. (9.8%) Alternaria alternate (7.3%), Chaetomium spp (2.4%) and Cladosporium spp (2.4%). These results were relatively similar to those results reported from other parts of the world, such as Cameroon [32], and Italy [33]. Dermatophytes are hyaline septated molds. The hyphae of these mycelial organisms penetrate the stratum corneum of the skin and nails. The fungal cells manufacture keratinolytic protease, which provide a means of entry into living cells [34].

Trichophyton rubrum was reported as the most common causative agent of onychomycosis in Senegal (53.6%) and France (85.1%) [35,36].

In the present study, Trichophyton tonsurans (34.5%) was the common pathogenic dermatophyte isolated followed by Trichophyton verrucosum (24%) and Trichophyton rubum (7%). These findings were not in agreement with those of another study, in which Trichophyton rubrum was the predominant spices (23%) followed by Trichophyton tonsurans (8.85%) and Trichphyton verrucosum (3.54%) [37]. This variation may be attributed to differences in geographical location, climatic conditions and population habits which may influence the distribution of dermatophyte spices. Other dermatophytic strain were isolated Trichophyton violaceum (13.8%), Trichophyton soudanense (10.3%), Trichophyton mentagrophytes (7%), and Epidermophyton floccosum (3.4%) of cases. These results support our observation that T.tonsurans and T verrucosum may play a significant role in onychomycosis in our region, possibly influenced by local environmental and occupational factors.

CONCLUSION

Onychomycosis is a common disease in our place and constitutes major health problem that profoundly affect the patient quality of life and functional activity. Onychomycosis was predominately seen in females with high frequency among the age group 30-39 years and DLSO was the most frequently clinical types found in both fingernails and toenails followed by PSO.

Although the causative agents of fungal infection are changing from time to time and from place to another, at present our study, showed that the common pathogenic organism causing onychomycosis in our area were candida albicanis and non-dermatophyte molds (Aspergillus nigra), followed by dermatophyte (T. tonsurans and T. verrucosum).

Statement of Human and Animal Rights

All the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 2008 revision of the Declaration of Helsinki of 1975.

Statement of Informed Consent

Informed consent for participation in this study was obtained from all patients.

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